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A study of Some Hormones and Antioxidant Systems Disturbances in Older Men

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Abstract

Ageing is a physiological phenomenon that manifested itself with disturbances of many homeostatic regulating mechanisms of the body . The present study was conducted and employed to investigate two major systems(hormones and antioxidant systems) that can be implicated in progress of aging .The total number of subjects included in the present study was fifty (50) healthy men and classified according to their ages into two groups, the first group included 25 younger men (control group) and their ages ranged between 21 to 30 years old whereas the second group included 25 older men and their ages were between 61 to 70 years old. Data obtained from this study indicated a significant decrease($p<0.05$) in the levels of thyroid stimulating hormone (TSH) which is associated with marked elevation ($p<0.05$) of triiodothyronine(T3) and tetraiodothyronine(T4) of older men when compared with younger men. In regard to levels of cortisol and testosterone hormones were significantly decreased ($p<0.05$) in aged men when matched with their counterparts of younger men. Concerning concentrations of malondehyde (MDA) ,a final product of lipid peroxidation, confirmed a remarkable elevation ($p<0.05$) in older men compared to younger men and these results were associated with significant lowering ($p<0.05$) in the activities of antioxidant components including glutathione peroxidase (GPX), reduced glutathione (GSH) and catalase . In addition , there are no significant correlations($p>0.05$) occurring among hormones(testosterone , T3) and glutathione peroxidase and of malondehyde . From these results ,one can be concluded that with ageing there are many disturbances and fluctuations of hypothalamic-adrenal and thyroid axis that accompanied with drop of essential antioxidant components that may be lead to suppress of defense against free radicals and the present study concluded that the changes occurring in studied hormones have not relations and effects on the antioxidant systems.

Key words: ageing , hormones , antioxidants .

دراسة بعض الاضطرابات الحاصلة لبعض الهرمونات والانظمة المضادة للأكسدة لدى الرجال المسنين

الخلاصة

تتميز ظاهرة التقدم بالعمر بحصول العديد من الاضطرابات خاصة في الميكانيكيات الفسلجية اللازمة لإدامة الاتزان البدني. وضعت الدراسة الحالية لبحث منظومتين يعتقد انهما ذات اهمية في حصول تقدم العمر وهما بعض الهرمونات ومضادات الاكسدة .شمل العدد الكلي لأشخاص الدراسة انتخاب خمسون رجلا من الاصحاء وقد قسموا حسب اعمارهم الى مجموعتين شملت المجموعة الاولى خمس وعشرين رجلا من الشباب(Younger men) تراوحت اعمارهم بين21- 30 سنه في حين شملت المجموعة الثانية خمس عشرون رجلا مسنا (Older men) تراوحت اعمارهم بين 61- 70 سنة . سجلت النتائج المستحصلة من الدراسة الحالية حصول انخفاض معنوي ($p<0.05$) في مستوى الهرمون المحفز للدرقية (TSH) وبنفس الوقت حصول ارتفاع معنوي($p<0.05$) في مستوى هرموني الدرقية (T3,T4)عند الاشخاص المسنين عند مقارنتهم مع الاشخاص الشباب , وبخصوص هرموني التستوستيرون (Testosterone) والكورتيزول (Cortisol) ,فقد بينت النتائج حصول انخفاض معنوي ($p<0.05$) في تراكيز كلا الهرمونين في الرجال المسنين عند مقارنتهم مع الرجال غير المسنين (الشباب) . سجلت قيم مركب المالونديهايد (MDA) ارتفاعا معنويا ($p<0.05$) لدى الرجال المسنين عند مقارنتهم مع الرجال الشباب في حين لوحظ حصول انخفاض معنوي في مستوى العوامل المضادة للأكسدة

مثل الكلوتاثيون بيروكسيديز (GPX) والكلوثايون المختزل (GSH) والكاتليز (Catalase) لدى الرجال المسنين عند مقارنتهم مع الرجال الشباب . كما بينت النتائج عدم حصول ارتباط معنوي ($p>0.05$) بين كل من هرموني T3 و هرمون Testosterone من جهة و مركب MDA وفعالية انزيم GPX من جهة اخرى .

من النتائج المستحصلة من هذه الدراسة يمكن الاستنتاج بانه مع تقدم العمر تحصل العديد من الاضطرابات والتقلبات في محاور جسم تحت المهاد و الدرقية والكظرية والتي تكون مصحوبة بانخفاض المنظومات المضادة للأكسدة والتي قد تودي الى ارتفاع مستوى الجذور الحرة والتي بدورها تسبب اضطراب للعديد من الوظائف الفسلجية للجسم على مستوى الاجهزة والخلايا .

الكلمات المفتاحية: العمر ,الهرمونات ,مضادات الاكسدة .

Introduction

With age , most if not all body homeostatic mechanisms become disturbed and tend to be progressively declined of their important and vital functions necessary to make the body systems act normally , the loss of efficiency of essential functions renders the body unable to adapt for different exogenous and endogenous stressful factors that finally lead to aggravate and exaggerate of many chronic diseases (Kumar *et al.*,2017). Ageing is a life phenomenon of all organisms especially mammals, this problem progress step by step and finally tend to damage several essential activities of body such as network of hormones, metabolism ,nutrition and growth (Jonas and Boelaert, 2015). Among systems affected with ageing is neuroendocrine system, the hypothalamic–pituitary axis and other peripheral endocrine glands become more affected with progress of age ,the primary responses and sensitivities of target receptors that localized on many cells become inactive and down regulation (Puzianowska- Kuznika, 2012). The hypothalamic-pituitary- adrenal is responsible for maintaining and regulation of many fluctuations and stressful conditions via variable levels of glucocorticoids secreted from adrenal cortex (McEwen, 1998) .In addition, hypothalamic- pituitary- thyroid axis is also subjected to many alterations during advanced age, the thyroid hormones undergo from dysregulation and fluctuations in their levels (Costa and Rosenthal ,1996 ; Slemer *et al.*, 2014).On the other hand, the reactive oxygen species (ROS) can be originated from either exogenous or endogenous factors , furthermore, there are many theories indicated that production of those free radicals resulted from metabolic pathways, in particular, mitochondria are implicated in deterioration effects of living tissues with progress of age (Adkins *et al* , 2017). The body has specific systems specialized in scavenging and delay the harmful effects of free radicals, these systems are composed from enzymatic and non- enzymatic antioxidant components (Lenas, 1998; Zsnagy,2001).

Materials and Methods

Subjects of the study

The present study was carried out in college of science for women of Babylon university and Marjan hospital from the period ranged from December 2016 to June 2017. The study included two different age groups of younger and older men. The total number of the subjects was 50 men and subdivided according to their ages into two groups , the ages of the first group of men (25 men) were ranged between 21 to 30 years old and were used as control group and the ages of the second group (25 men) were 61 to 70 years old . All subjects were volunteered and recruited from Babylon university and public health laboratory. All subjects of the study were selected carefully to exclude the subjects (men) who complain from common chronic diseases and other bad habits such as smoking and drug abuse. The principle

questions were directed to the subjects about essential their health criteria and life style to explain the subjects have a complete fitness to include them in the present study.

Collection of blood samples

All blood samples were collected during morning between 8 to 10 a.m. Before collection , the subject asked to rest on a chair for at a least 10 minutes , and then the anticubital vein of left arm are mostly employed. The site of collection were cleaned with alcohol (70%) and wormed to improve blood circulation and engorge the targeted vein . A tourniquet was applied at 7 cm above site of collection . A syringes with 23 gauge were used and adequate blood samples withdrawn and then transferred to gel plain tubes (without anticoagulants) to permit the blood to coagulate . After completing coagulation , the samples transferred for centrifugation at 3000 rpm for 15 minutes and then serum was aspirated by micropipettes and kept by eppendroff tubes . The serum samples were kept in a deep freeze (- 20 ℃) to perform the future biochemical analyses .

A- Estimation of hormones

Measurements of thyrotropin hormone (TSH), triiodothyronine hormones (T3), tetraiodothyronine hormone (T4) ,testosterone hormone and cortisol hormone these hormones are determined by ELISA and Minividas technique (according to kits of Biomerixue company) .

B-Determination of antioxidants

1- Assay of catalase activity

Catalase is an enzyme that catalyses the decomposition of hydrogen peroxide molecules (H2O2) to give final products (H20 and O2). Catalase activity is measured according to previous method reported by Aeobi (1974). The decomposition of H2O2 is followed directly with decrease in the absorbance of samples at 240 nm wave length . The difference in absorbance per unit of time represented the measurement of catalase activity .

2-Activity of glutathione peroxidase

Glutathione peroxidase is determined according to method invented by Paglia and Valetin (1967).

3-concentrations of reduced glutathione

5.5-dithiobis (2-nitrobenzoic acids) (DTNB) is a disulfide chromogen that is immediately reduced by the presence of sulfahydral group of reduced glutathione (GSH) in serum sample to produce intensely yellow color . The absorbance of reduced chromogen is estimated at 412 nm that directly proportional to GSH concentrations (Burtis and Ashwood , 1999) .

4-Level of malondialdehyde (MDA) concentrations

The concentrations of MDA is based on the ability of MDA to react with thiobabituric acid (TBA) producing final product(MDA- TBA2) and its absorbance is read at 532nm.(Guide and Shah, 1989)

C- statistical analysis

All results of the present study are showed as means ± standard deviation (SD) and the results are analyzed by using SPSS program. The differences among different groups are performed by using Student t- test and $p< 0.05$ represented the lowest significant limit (Daniel, 1999) .

Results of the study

The results that are obtained from the present study are illustrated in the following table which explained below and these results have a significant increase ($p<0.05$) in the levels of thyroid hormones(T3 and T4) associated with decrease levels of TSH in older men in a comparison with younger men. In regard to levels of cortisol and testosterone hormones were significantly drop ($p<0.05$)in older men. Furthermore, values of antioxidants (GPx,GSH, Catalase) showed a remarkable fall ($p<0.05$) in older men. In contrast, concentrations of(MDA), a final product of lipid peroxidation, indicated a significant increase($p<0.05$) in aged men higher than of younger men.

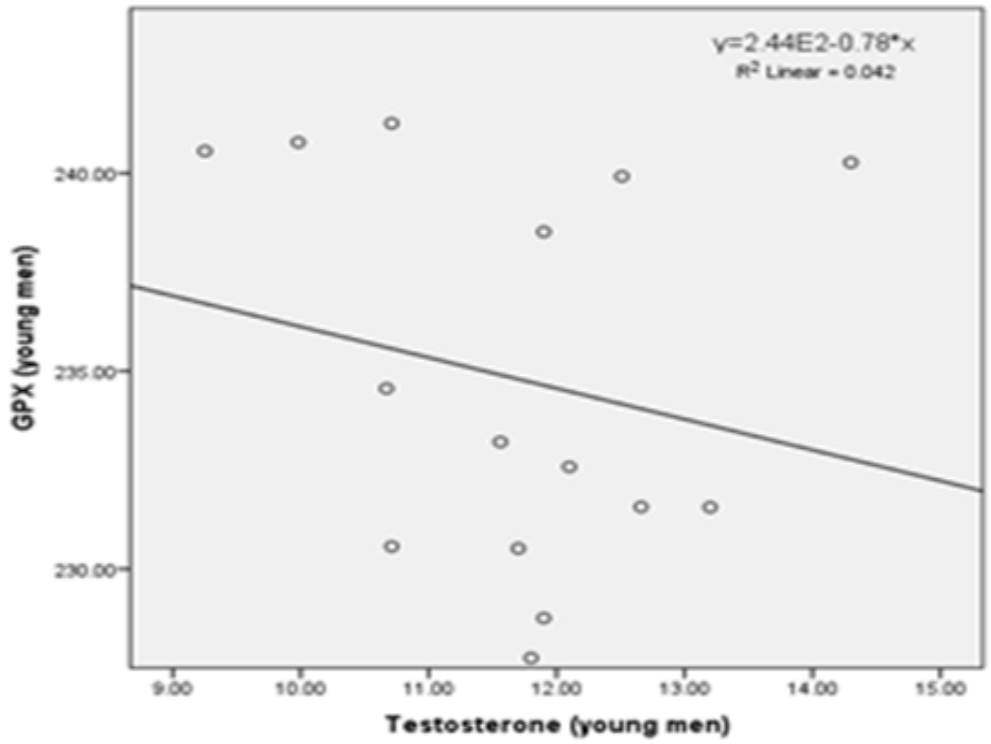
Table :- Shows the results of hormone levels (thyroid stimulating TSH MIU/ml, Triiodothyronine T3ng/ ml, Tetraiodothyronine T4 ug/ml, Cortisol ng/ml, Testosterone ng/ml) and oxidant- antioxidants activities (Malondialdehyde MDA umol/ml ,Glutathione peroxidase GPX U/L , Reduced glutathione GSH mg/dl, and Catalase K/ml) in younger and older men .

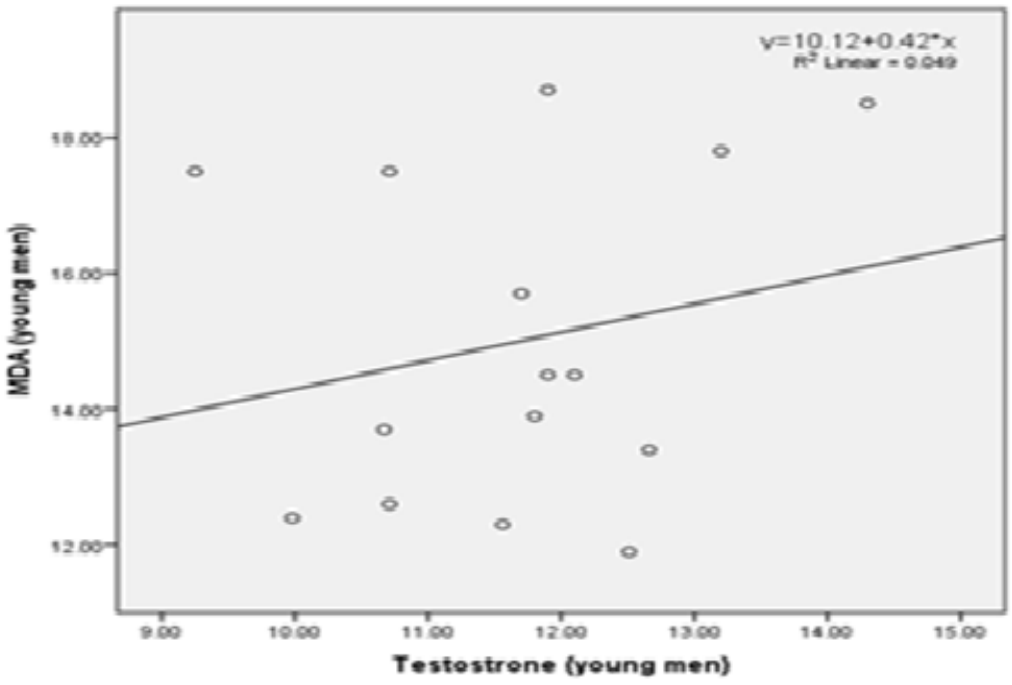
Parameters / Groups	Younger men (21-30 years)	Older men (61-70 years)	P value
TSH (MIU/ml)	2.66± 0.33	1.65 ±02*	P<0.05
T3 (ng/ml)	0.947± 0.03	1.75 ±0.35*	P<0.05
T4 (µg/ml)	10.4 ± 1.33	12.18 ± 0.61*	P<0.05
Cortisol (ng/ml)	115.45± 4.16	95.22±3.85*	P<0.05
Testosterone(ng/ml)	11.66±1.27	7.84± 1.01*	P<0.05
MDA (µmol/ml)	14.99±2.42	39.80±5.98*	P<0.05
GPX (U/L)	234.82±4.86	176.20±15.09*	P<0.05
GSH (mg/dl)	46.96±4.15	24.69±2.76*	P<0.05
Catalase(K/ml)	3.77±0.96	1.24 ±0.55*	P<0.05

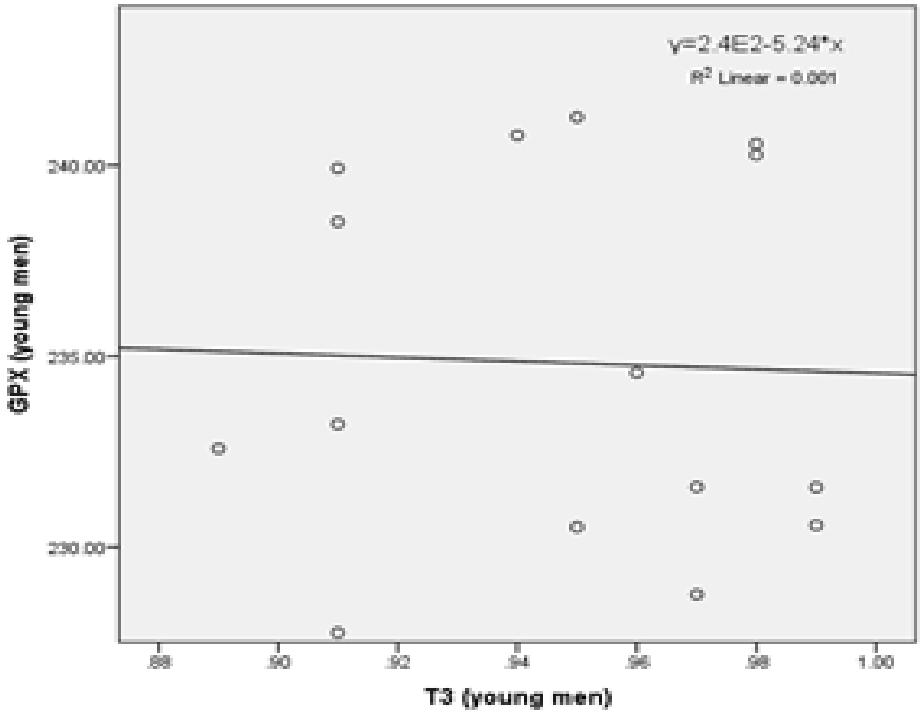
-All values are means ± standard deviation

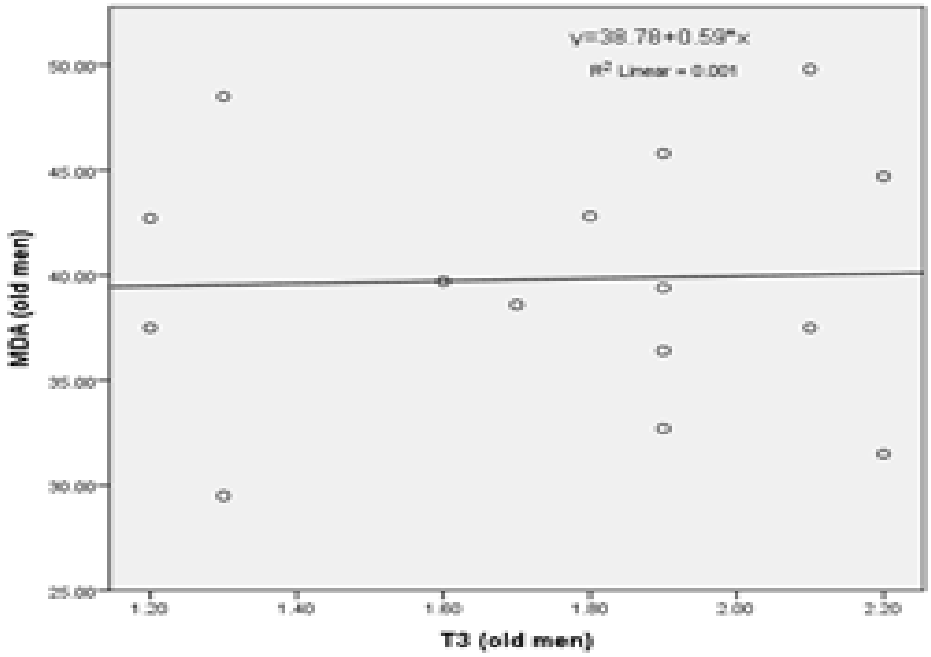
-Means with mark * are significantly different($p<0.05$).

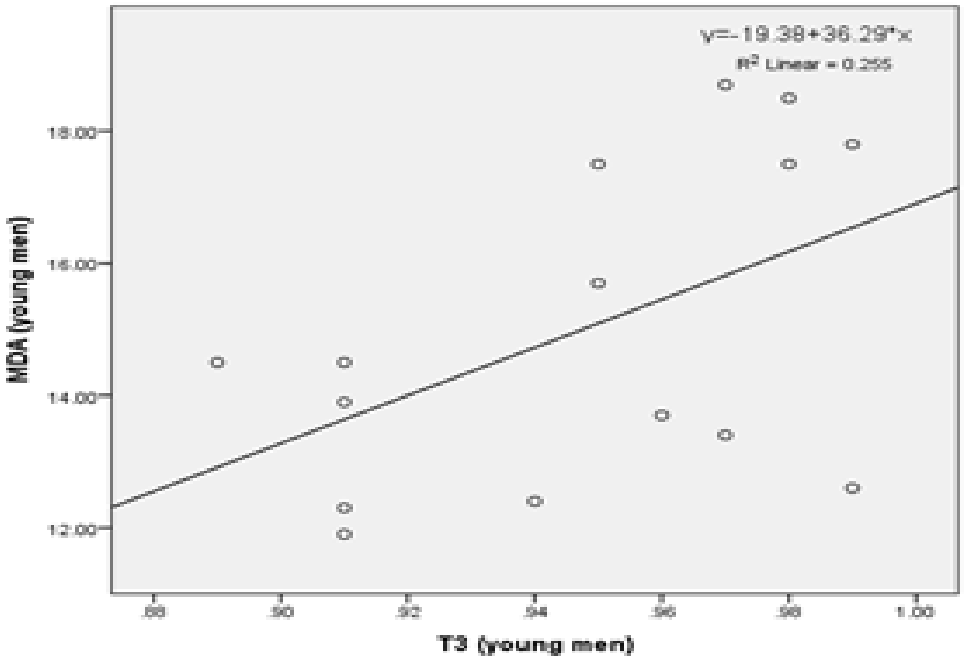
-The following figures refer to correlations among T3 and testosterone hormones with MDA and GPX in both younger and older men:-

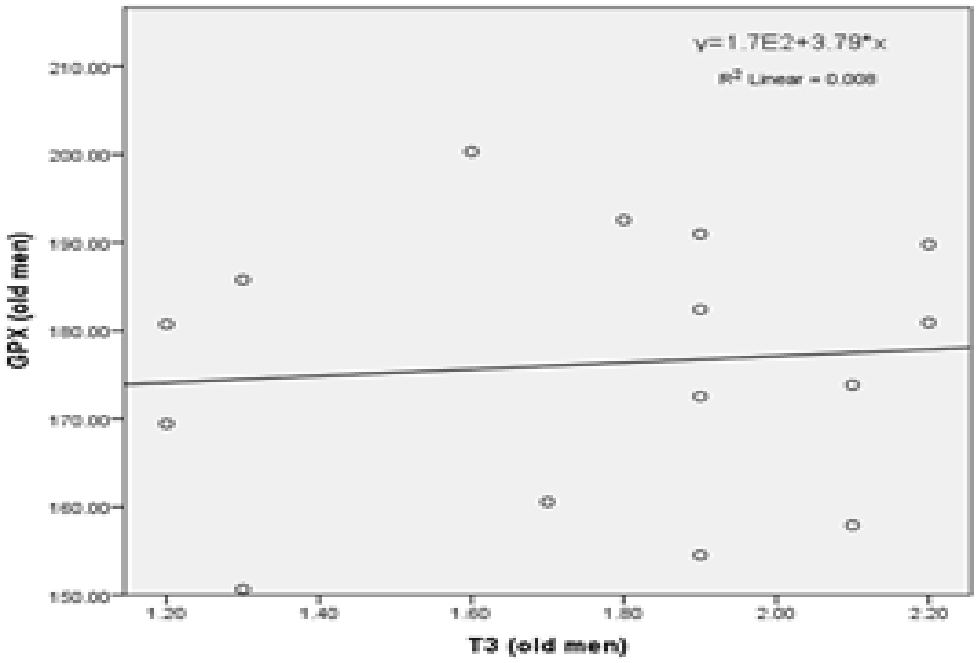

y=2.44E2-0.78*x
R² Linear = 0.042
GPX (young men)
240.00
235.00
230.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
Testosterone (young men)

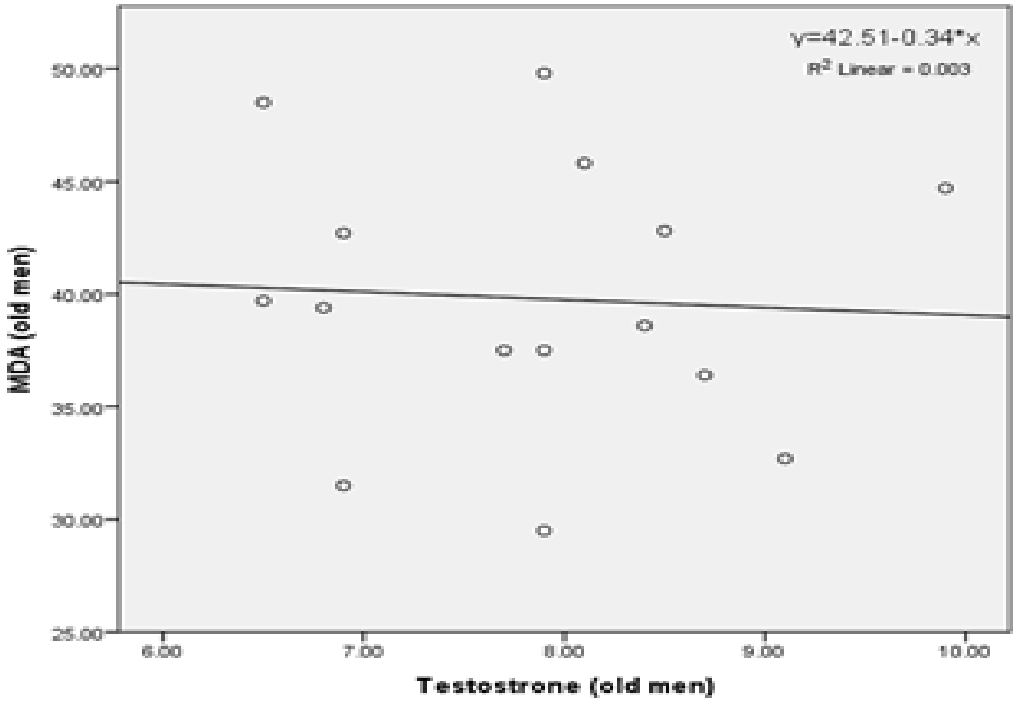

y=10.12+0.42*x
R² Linear = 0.049
MDA (young men)
18.00
16.00
14.00
12.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
Testostrone (young men)

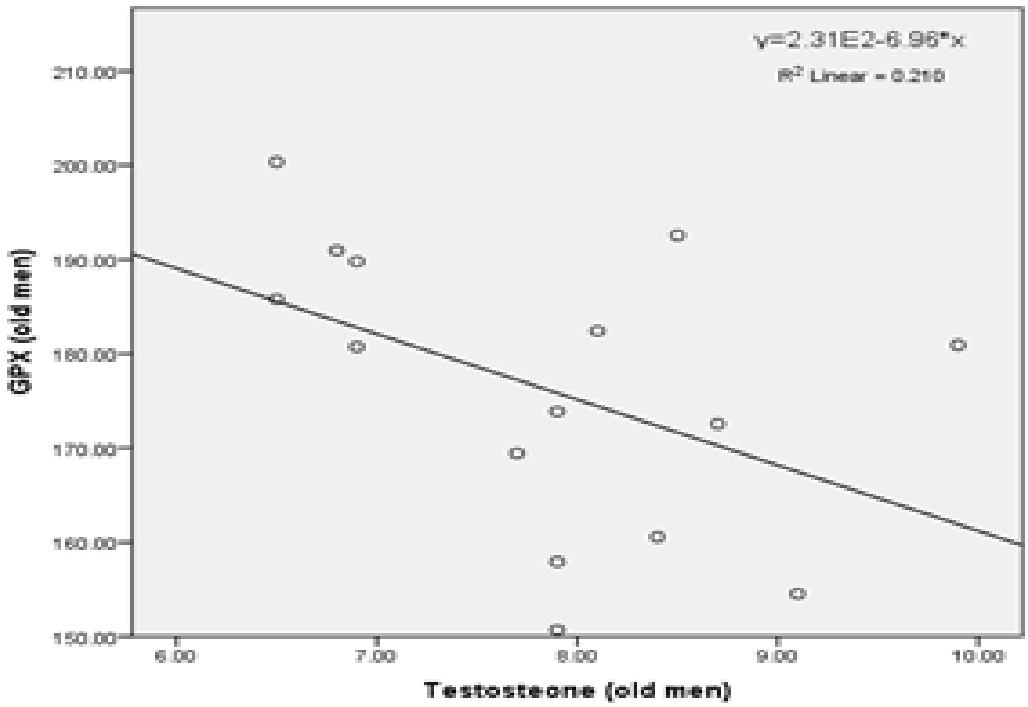

Y=2.4E2-5.24*x
R² Linear = 0.001
240.00
235.00
230.00
GPX (young men)
.88
.90
.92
.94
.96
.98
1.00
T3 (young men)


Y=38.78+0.59*x
R² Linear = 0.001
50.00
45.00
40.00
35.00
30.00
25.00
MDA (old men)
1.20
1.40
1.60
1.80
2.00
2.20
T3 (old men)


y=-19.38+36.29*x
R² Linear = 0.255
MDA (young men)
18.00
16.00
14.00
12.00
.88
.90
.92
.94
.96
.98
1.00
T3 (young men)


y=1.7E2+3.79*x
R² Linear = 0.008
GPX (old men)
210.00
200.00
190.00
180.00
170.00
160.00
150.00
1.20
1.40
1.60
1.80
2.00
2.20
T3 (old men)





The results obtained from the present study indicated the following data:-

1-There is a negative non- significant ($p>0.05$) correlation between testosterone and GPX activity in younger men .

2-There is a positive non-significant ($p>0.05$) correlation between testosterone and MDA concentration in younger men.

3-There is low negative non -significant ($p>0.05$) correlation between T3 and GPX activity in younger men.

4-There is a low positive non- significant ($p>0.05$) correlation between T3 and MDA Concentration in older men.

5-There is positive non- significant ($p>0.05$) correlation between T3 and MDA concentration in younger men.

6-There is no correlation between T3 and GPX in older men.
7- There is low negative non- significant ($p>0.05$) correlation between testosterone and MDA level in older men
8- There is a negative non- significant ($p>0.05$) correlation between testosterone and GPX in older men.

Discussion

Despite increase health care programs and requirements of all modern life style such as nutritional demands and several facilities of life affairs particularly in developed countries , the ageing phenomenon or ageing problem remains the fate of all humans .The exact and causative factors responsible for this problem remain obscure . Many theories and suggestions tried and offered multiple explanations , but, most if not all of previous and recent efforts brought more attentions toward oxidative stress, so that the present study involved the investigations to estimate of some hormones that fluctuated throughout the age and perhaps their relations with antioxidant systems of aged men compared to their counterparts of younger men.

Thyroid hormones

In this study , it found that the levels of T3 and T4 are significantly (($p< 0.05$) higher in older men than of younger men , but on the other hand , the levels of TSH are significantly ($p< 0.05$) decreased in aged men .

In elderly human , the thyroid gland functions are subjected to many changes in their hormone production , metabolism and activities, moreover, there are a wide reference ranges have been found in the levels of both thyrotropin (TSH) and thyroid hormones (T3 and T4) between younger and aging population (Aggarwal and Razi , 2013). As individuals become aged , their body systems begin from cells and their cellular components have undesirable changes that they target especially endocrine organs ,those abnormal changes can affect synthesis or secretion of hormones as well as target tissues having their receptors that are specialized to those hormones ,In addition, it was found that FSH and LH hormones seem more affected and most of these changes were found to be associated with peripheral degradation or resistance to thyroid hormones (World Population Prospects , 2006).

Some previous results confirmed that ageing is implicated to increase prevalence of sub- clinical hypothyroidism that associated with increased levels of TSH and free T4 particularly in individuals over 60 years old (Biondi and Cooper,2008) .

The present results are also inconsistent with previous study of Margi *et al*. (2002) who showed that thyroid gland appears normal and with no significant abnormalities in its size and appearance , but on other hand , they confirmed increased tissue density although the iodine trapping mechanism remains with normal limits, finally ,these observations indicated increase of TSH levels associated with down regulation of T3 and T4 , these observations appearently conflict with results obtained from this study and may be affected because of the local and special factors of those population.

In regard to response of central nervous system to the repeated stress , it is that found changes occurred with progress of age through perform study on experimental animals (rats), the findings of this study indicated increase stress with advanced age resulting in up regulation of responses by serotogenic neurons to stress related with age (Yamaguchi *et al.,* 2016) so, the presented results can be explained there are some problems affected hypothalamic- pituitary thyroid/adrenal axis at a level thyroid or

adrenal cortex . From physiological point view, that the thyroid hormones have abilities to produce ROS through their functions involve increasing the basal metabolic rate and accelerating respiratory chain rate (Villanueva *et al.,* 2013) and enhancing the production of ROS that confers the prove increase oxidative stress .

Cortisol

Levels of cortisol indicated a significant lowering ($p<0.05$) in older men. Study of Wong and Shobo (2017) found that older men have high risk for development of hypothalamic–pituitary- adrenal dysfunction than of younger men . Among hormones, Cortisol represents the common hormone that has fluctuations and diurnal variations during the day ,the cortisol peak appear after 20 to 30 minutes from beginning of wakefulness and then return to lower limits in the remaining time of the day (Fries *et al.,* 2009).

The axis of HPA and thyroid become less effective when individuals reach the advanced age (Chahal and Drake, 2007). Previously, it is well established that younger men is more reactive to stressful conditions than of older men because younger men have little cognitive behaviors in a comparison with older men to deal with stress states (Neupert *et al* ., 2007).

Data of the present study are inconsistent with study of Rehman and Masson (2001) that established dysregulation of negative feedback mechanism to stress conditions including HPA axis and they confirmed that adrenocorticotropin and cortisol hormones can remain with normal lower limits in younger individuals than older men and who suggested that a high level of activation and impairment of adequate responses is less active in older men.Larsson *et al* . (2009) showed different results and confirmed increase secretion of cortisol with progress of age . Indeed , the increase of HPA axis reactivity in response to stressful conditions in older ages continue for long time in contrast to younger ages (Bazhanova *et al* ., 2000) .

Alemany (2012) in his study confirmed the facts that sexual hormones (androgens and estrogens) inhibit inflammatory processes and tissue responses to actions of glucocorticoids ,with age , there is a down regulation of androgen and estrogen lead to increase factors that can mediate by increasing levels of glucocorticoids .Previous research that has carried out on lab animals to explain how the advanced age can affect adrenal cortex , it is found that zona fasculata and reticularis become hypertrophy in aged animals with the size and number of their intracellular organelles, these finding are attributed to increase the levels of adrenocorticotrophic hormone and cells of adrenal cortex unable to uptake and utilization of cholesterol molecules, and concluded there is a drop in the levels of glucocorticoids in older animals (Rebuffat *et al.,* 1992) .

Most if not all, older human undergo from many neurodegenerative diseases at different levels and these diseases can be masked , a higher levels of glucocorticoids , in particular, cortisol are found significantly associated with hippocampal abnormalities and impairment of memory . such alterations in aged men can lead to prolonged secretion of cortisol and disturbances of circadian rhythm (Lupien *et al.,* 1998). The present data appear conflicted with results of Van *et al* . (1996) that established HPA axis affect with minor changes that related with aging and levels of glucocorticoids remain with normal values or moderately increased .

It is well documented in experimental studies that injection of animals with cortisol did not suppress or activate antioxidant systems and these studies concluded that high levels of cortisol can produce negative physiological events and not affect oxidant – antioxidant systems (Gauvin *et al* ., 2017) .

Testosterone

It is not surprising ,that ageing affects androgen hormone (Testosterone) , the drop of serum testosterone in older men is called late hypogonadism (Perheentupa and Huhtaniemi, 2009) .The previous study indicated decrease sexual activity with ageing and accompanied with drop of total and free testosterone levels (Oconnor *et el.,* 2011; Orwoll, 2016).

A lot of biochemical changes are associated with decreased levels of testosterone in aged men , there is an increase of sex hormone binding proteins in blood circulation lead to decrease of free testosterone and the lowering levels of this hormone can impair negative feedback mechanism on hypothalamic – pituitary that exert by hormone self and lead to increase the gonadotropin (FSH and LH) levels (Lunenfeld *et al.,* 2015). The drop of essential sexual hormone (testosterone) is correlated with many physiological changes in older individuals including hyperinsulinemia, increased triglycerids and low density lipoproteins, decreased high density lipoproteins, and neurophysiological changes (Bhasin *et al.,* 2010) . Recent study indicated that treatment of old men with testosterone leads to support and improve several sexual indicators including sexual activity, sexual desire , and errectil functions (Cunnigham *et al.,* 2016) .

Concerning the relation of testosterone with oxidative stress , previous researchers confirmed apposite and different outcomes , it is documented that testosterone can induce oxidative stress and observed decrease life span of red blood cells because they become less resistance to lyse generating by injection of testosterone to those experimental animals (Alvarez *et al.,* 2007) . In contrast , it is found that the drop of testosterone depress the expression of intratesticular antioxidants that in turn causes to suppress of spermatogenesis and extensive apoptosis of germ cells (Aitken and Roman, 2008). Also, recent further study confirmed that testosterone in healthy normal men can provide defense and neuroprotection to prevent incidence of neuro degenerative diseases , but in older men , there is increase of oxidative stress and low levels of testosterone , they become more susceptible to neurodegenerative diseases (Homes *et al.,* 2016).

Antioxidants

The results of the present study showed clearly that antioxidant components undergo sharp decrease accompanied with a high level of lipid peroxidation products (MDA) in aged men. In recent years, many studies and efforts were focused to understand and explain the relation of oxidative stress and progress and incidence of ageing and chronic diseases (Verrugio *et al.,* 2016). The present data are supported by Ide *et al.* (2002) who confirmed that men have higher levels of oxidative stress than of women.

In addition , recent study improve an understanding to explain how ROS have many deteriorations effects on many body organs and exert their harmful effects on mitochondria which become more subjected to much damage by ROS associated with lowering ATP production when human become aged (Abbas *et al.,* 2017).Many of researches and studies are conducted to investigate the relationships between mitochondrial DNA (mtDNA) mutations and oxidative stress that take part in progress and development of ageing, mtDNA mutations are mostly originated from loss repairing mechanisms having nuclear DNA , according to these facts ,there is no doubt that ageing is associated with accumulations of several mutations in mtDNA as a result of ROS (Pinto and Moraes, 2015).

Mitochondria and their components of respiratory chains represent the major pathway in generating reactive oxygen species as side products, respiratory enzymes become more exposed to these toxic compounds , moreover, reactive nitrogen species also are produced and synergized with ROS to increase injuries and damage of mitochondrial components causing several degenerative alterations that in turn lead to develop ageing (Murphy, 2009; Rju, 2014).

Although the toxic effects of ROS , they have some benefits and important to body functions such as killing of pathogens by immune system and intracellular signaling when they are presented at lower levels , but excess levels of ROS causes damage and deterioration to many organs and system rendering the body loss many vital functions and aging development (Jevas,2016). mtDNA and nuclear DNA become more influenced by ROS , many modifications can occur in structures of DNA because of ROS and these modifications including break DNA strand , purine and pyrimidine changes . Also , many mutations hit the genomic potential such as point , translocation, and binding with other proteins (Girotti, 1985 ; Cui *et al.,* 2012).

In conclusion , the results that obtained from this study indicated that there are many physiological disturbances occurring in hypothalamic adrenal and thyroid axis associated with fall levels of testosterone hormone .These hormonal dysfunction are also accompanied with down regulation of antioxidant systems and according to these data can be suggested the ageing is a physiological phenomenon resulted from accumulation of different abnormalities of homeostatic mechanisms responsible for adjustment of many body functions.

References

-Abbas ,G.;Salman ,A.;Rahman ,S.U.;Ateeq,M.K.;Usman,M.;Sajid,S.;Zaheer, Z.& Younas,T.(2017).Aging mechanisms: linking oxidative stress,ob Esity and inflammation.Matrix Scince Med.(MSM),1(1):30-33.

-Adkin,A.G.;Yanez,Y.;Peris-Diaz,M.D.;Calabria,I.;Sadoval,J.(2017).Epigeneti Cs and oxidative stress inaging.Oxid.Med.Cellular Long.,2017:1-8.

-Aebi,H.(1974).Catalase.Methods of Enzyamtic Analysis 2nd Ed(Bergmeyer H) Academic Press,New Yourk P:673-677.

-Aggarwal,N.&Razvi,S.(2013).Thyroid and aging ,of the aging the thyroid?An evidence based analysis of the literature.J.Thyroid Res.,213:1-8.

-Aitken,R.J. & Roman,S.D.(2008).Antioxidants system and oxidative stress In the testes .Oxid.Med.Cell Longev.,1(1):15-24.

-Alvarez,C.A.;Bertrand,S.;Faivre,B.;Chastel,O.;& Sorci,G.(2007).TestosteronE and oxidative stress.The oxidative stress.The oxidation handcip Hypothesis.Proc.Biol.Sci.,274:819-825.

-Alemany, M.(2012).Do the interactions between glucocorticoids and sex h Ormones regulate the development of the metabolic syndrome?.Endocrinol., 3:1-13.

-Bazhanova, E.D.; Chernigovskaya,E.V.;&Danilova, O.A.(2000).Different path Ways of neurohormonal hypothalamic control of adrenal cortex fu Nction in young and old rats . Mech.Aging,118:91-102.

-Bhasin,S.;Cunningham,G.R.;& Hayes,F.T.(2010).Testosterone therapy in mEn with androgen deficiency syndromes: an endocrine society gui Deline.J.Clin.Endocrinol.Metabol.,95:2536-2559.

-Biondi,B.& Cooper,D.S.(2008).The clinical significance of subclinical thyrio D dysfunction .Endocrinol.Rev.,29:76-131.

-Burtis,C.A.& Ashwood,E.R.T.(1999).Text Book of Clinical Biochemistry.3rd Ed.Philadelphia,WB Saunders:45.

-Cai,H.;Mcneilly,A.S.;Luttreell,L.M.;&Martin,B.(2012).Endocrine function in aging. Int. J. Endochrinol.,10:1-3.
-Chahal,H.S. & Drak,W.M.(2007).The endocrine system and aging.J.Path.,2 11(2):173-180.
-Costa,C.V.M.& Rosenthal,D.(1996).Effect of aging on thyroidal and pituit Ry T 4-5 deiodinase activity in female rats.Life Sci.59(8):151-152.
-Cui,H.; Kong, Y.; & Zhang,H.(2012).Oxidative stress,mitochondrial dysfunctiOn, and aging .J.Signal.Transduct.,51:3-13.
-Cunniigham, G.R.; Shield, A.J.; Rosen, R.C.; Wang, C.; & Bhasin, S. (2016). Testos Erone treatment and sexual function in older men with low testost Erone levels. J. Clin.Endocrinol.Metab.,101:3069-3104.
-Daniel,W.W.(1999).Biostatistics: a foundation for analysis in health scienc 7ed .J. Wily Philadephia.P(8).
-Frie, E.; Dettenborn,L.; & Kirschbaum, C. (2009). The cortisol awakening resn Ponse (CAR): facts and future directions.Int.J.Psych.,72:67-73.
-Gauvin, K.B.; Peiman, K.S.; Larsen, M.H.; & Cook, S.J. (2017).Short term and lN oxidative stress in juvenile brown trout. J.EP. Biol.,220:1693-1700.
-Girotti,A.W.(1985).Mechanisim of lipid peroxidation.J.Free.Radicals.Biol. Med.,1:87-95.
-Guide, B. & Shah, S.V. (1989). Enhanced in vivo H2O2 generation by vat kidNey in glycel-induced venal failure.American. J. Physiol.,1257:440-444.
-Holmes,S.Singh,M.;Su,C.;& Cunningham,R.L.(2016).Effects of oxidative s Tress and testosterone on proinflammatory signaling in a female S rat dopaminergic neuronal cell line.Endocrinol.,157:2824-2835
-Ide, T.; Tsutsui, H.; Ohashi, N.; Hayashidani,S.;&Suematsu,N.(2002).Greater Oxidative stress in healthy young men compared with premenop Ausal woman. Arter. Throm. Vasc. Biol., 22:438-442.
-Jevas, O.C. (2016).The role of reactive oxygen species and antioxidants in Oxidative stress . Inter. J. Res. Pharm. Bioscie.,3(6):1-8.
-Jonas, M.; Kurylowics, A.& Kuznika, M.(2015).Aging and endocrine systemPostepy PN auk Medyczych,T.XXVIII,Nr.7;451-456
-Jonas,C.M. & Boelaert,K.(2015).The endocrinology of ageing :Amini-Revi Ew. Gerontology, 61:291-300.
-Kumar, D.R.; Shaik, R.B.; & Sundari,H.(2017).A reviw on effects of aging on dhatus.Inter.J.Ayurvedic and Herbal Med.,7(1):2436-2441.
-Larsson, C.A.; Gullberg, B.; Rastam, L.; & Lindbald,V. (2009). Salivary cortisol Differs with age and sex and shows inverse association with WHR in Swedish women .A cross sectional study. BMC endur.Disord.9:1-11.
-Lenaz, G. (1998).Role of mitochondrial in oxidative stress and aging .BioChimica Biophysica.Acta.,1366(1-2):53-67.
-Lunefeld, B.; Mskhalaya,G.; & Zitzmann, M. (2015).Recommedations on the Diagnosis, treatment,and monitoring of hypogonadism in men.AgIng Male, 2015:1-11.
-Lupien, S.J.; Leon, M.D.; Santi, & Convit, C.T. (1998).Cortisol levels during huMan aging predict hippocomple atrophy and memory deficit.NatUre Neuroscience,1(29):69-73.
-Magri, F.; Muzzoni, B.; & Cravello, L. (2002).Thyroid function in physiologica Aging and in centenarians :possible relationships with some nutriTional markers.Metabolism ,51:195-109.

-McEwen, B.S. (1998). Protective and damaging effects of stress mediatorsNew Engl. J. Med., 338:171-179.
-Murphy, M. (2009). How mitochondria produce reactive oxygen species. BIochem.J.,417:1-13.
-Neupet, S.D.; Almedia, D.M.; & Charles, S.T. (2007).Age differences in reactiVity to daily stressors:the role of personal control.J.Gerontology: Psych.Sci., 620,216-225.
-Oconnor, D.B.; Lee, D.M. & Corona, G.(2011).Sex hormones and sexual funCtion in middle-aged and older European men .J.Endocrinol.Met96:1577-1578.
-Orwoll, E.S. (2016).Establishing a frame work –D0es testosterone suppleMentation help older men. N. Engl. J. Med., 374:1-7.
-Paglia, E.D.; & Valetin, W.N. (1967).Studies on quantitative and quantitativOf erythrocyte glutathione peroxidaes.J.Lab.Clin.Med.,70:158-169
-perheentupa,A.& Huhtaniemi,I.(2009).Aging of the human ovary and teStis. Mol. Cell. Endocrinol.,13:1-12.
-Pinto, M.& Moraes,C.T.(2015).Mechanisims linking mt DNA damage and Aging. Free Radic. Biol.Med.,85:250-258.
-Poulse, N.& Raju,R.(2014).Aging and injury:alteration in cellular energetiCs and organ functions in aging.Dis.,5:101-108.
-Puzianowska-Kunika,M.(2012).Endokrynologia Okresu Starzenia.(W) MilEwicz A (red):Endokryologia Klinicza. Polskie Towarzystwo Endokry Noloiczne, wroclow,111:223-247.
-Rehman, H.U. & Masson,E.A.(2014).Neuroendocrinology of aging.Age Ag Ing, 30:279-289.
-Rebuffat, P.; Bellni, A.S.; Rocco, S.; Andreis, P.G.; & Nussdorfer, G.G. (1992). Th E effects of ageing on the morphology and function of the zone faSculata and zona reticularis of rat adrenal cortex.Cell and Tissue REs.,270(2):256-272.
-Slemer,C.;Olesen,J.B.& Hansen,M.L.(2014).Subclical and overt thyroid dYsfunction and risk of all-cause mortality and cardiovascular eveNts :a large population study. J. Clin. Endocrinol. Metab., 99(7):237
-Van, C.E.;Leproult,R.;&Kupfer,D.J.(1996).Effects of gender and age on thE levels of circadian rhythmicity of plasma cortisol.J.Clin.Endo Crinol.,81:2468-2473.
-Verrugio, C.C.; Ortega, M.R.; Mosqueira, M.; & Simon, F. (2016). Oxidative strEss in disease and aging :mechanisim and therapies.J.Oxid.MEd.Cellular Long., 2016:2-5.
-Villanueva,I.;Alva-Sanches,C.;& Rosado,J.P.(2013).The role of thyroid hoMones as inductors of oxidative stress and neurodegeneration. Oxid. Med.Cell Long., 2013:1-15.
-Wong, J.D.& Shobo,Y.(2017).The influences of daily stressors on morningCortisol levels in middle life and older retirees.The moderating Roles of age and gender. J. Aging Health,29(5):858-879.
-World population prospects (2000).Executive summary,United nations.Department of economic and social affairs,population division.
-Yamaguchi, N.; Nakajima, N.; Okada,S.; &Yuri,K.(2016).Effects of on stress-Related responses of serotonergic neurons in dorsal raphe nucl Eus of male rats. Neurobiology of stress,3:43-51.
-Zsnagy, A(2001). On the true role of oxygen free radicals in living state,ag Ing